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DEVELOPMENT IN *PODOCARPUS NIVALIS* IN RELATION TO OTHER PODOCARPS. I. GAMETOPHYTES AND FERTILIZATION.

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This paper continues studies already published on development and embryogeny in podocarps with special reference to *Podocarpus andinus* and *Saxe-Gothaea conspicua* (Looby and Doyle, 1939, 1944 *a*, *b* ; Doyle and Looby, 1939).

Development in the genus *Podocarpus*, though it has received some attention, has not yet received the attention it deserves. The genus is one of the largest and most important in the family of the *Podocarpaceae*, a family of southern conifers which is of interest not only as the source of many excellent timbers but because it has probably developed independently from the northern conifers from quite early times (cf. Florin, 1940). Divided as it is into several rather distinct sections showing quite marked differences in vegetative and strobiloid features, a proper understanding of developmental tendencies shown within the genus can only be gained by a full investigation of representative species from each section ; and such a fuller knowledge of gametophytes and early embryogeny over a wider range might well help in the many vexed problems in taxonomy which the genus presents.

In the first decade of this century the question of the origin and development of the pollen grain in podocarps, with particular emphasis on the origin of the characteristic multiple prothallial cells, received considerable attention. This aspect is of no immediate interest here and full references are available in Schnarf's (1933) monograph. Apart from this, contributions to general development and embryogeny in the genus have been made by Coker (1902), Stiles (1912), Sinnott (1913), Buchholz (1936, 1941), Tahara (1941), and Looby and Doyle (1944 a, b). The account of *Podocarpus andinus* given by these latter authors is reasonably full, and is indeed the only full account available of any single species of *Podocarpus*. Tahara's paper deals only with embryogeny in *P. macrophyllus* and *P. nagi*, and, though somewhat inadequate, is of value for the facts presented on the embryogeny of *P. nagi*.

Buchholz's work, while of great intrinsic importance and touching on species of every section, is essentially limited to late embryogeny only, proembryo stages being in most cases only guessed at by inference. It holds much of interest for later comparison with corresponding stages in *P. nivalis*. Stiles' paper deals with the whole family, and, being largely anatomical and theoretical, contributes but little of value for developmental study. As far as *Podocarpus* is concerned it is practically limited to a summary account of the gametophytes of *P. macrophyllus* with a few words on *P. latifolius* and *P. nagi*. Sinnott's paper deals with the whole genus, and, while largely devoted to the structure of the ovulate branchlets and ovules, gives briefly some account of development in two species of the *Stachycarpus* section, one of *Dacrycarpus*, and three of *Eupodocarpus*. This latter part of his work, which includes reference to *P. nivalis*, is a summarized account of a general course of development which is said to be essentially the same in the three species quoted. Details of *P. nivalis* available from the present study show that this aspect of Sinnott's work is accurate enough though somewhat inadequate, but his further statements on embryogeny are quite superficial. Coker's paper though published more than fifty years ago, has many points of interest. The first paper published on any podocarp, and one of the first modern papers on any conifer, it is limited to one species of *Podocarpus*. There is some little doubt as to the identity of the species, but Buchholz (1941), who had an opportunity of examining the material, states, and probably correctly, that the species was *P. macrophyllus* var. *Maki*. It was in any case a member of the *Eupodocarpus* section. In it, as well as a general account of the female gametophyte to which the papers of Sinnott and Stiles add little, there is the first reference to that extreme inequality of the male cells which seems to be a characteristic of all species of *Podocarpus*, and also to the binucleate condition of the early embryo cells of the proembryo, a feature now known to be a characteristic of podocarps in general. The actual stages in proembryo development, however, were obviously not properly understood by Coker, and, even as far as these accounts go, there are certain discrepancies between his account of *P. macrophyllus* and that given by Tahara, and between both and Sinnott's account of the species investigated by him.

In the section *Eupodocarpus*, to which *P. nivalis* belongs, there is thus available, excluding the work of Bucholz on later embryogeny, three accounts of one species and a generalized account of three others, one of which is actually *P. nivalis*. All these however, as appears from the survey above, only yield a general account of the gametophytes ; the early embryogeny is left uncertain and obscure. Reference to Johansen's (1951) recent monograph on plant embryology only emphasises this obscurity. As then there were available and convenient in the National Botanic Gardens, Glasnevin, Dublin, an ovulate and a staminate plant of *P. nivalis* it was felt advisable to attempt for at least one eupodocarp what had been done for the stachycarpean *P. andinus*, though perhaps not quite so intensively. It was obviously necessary to try to follow the embryogeny as closely as possible, but in view of the fact that the accounts of the gametophytes are now up to fifty years old, it was also felt advisable to confirm, and if necessary to emend or extend, our knowledge of the earlier stages. Comparisons are made with known developmental phases in other podocarps.

Though the two specimens were reasonably close natural pollination could not be relied upon. The ovulate plant was however quite successfully pollinated by shaking and scattering over it branches from the other when the pollen was ripe. Pollen was available in quantity but, naturally on a small shrub such as *P. nivalis*, ovules were in relatively short supply. Intensive collection was therefore limited to important critical stages the dates of which had been approximately determined from previous experimental pollinations. Certain less important or routine phases are passed over lightly, or even omitted, thus avoiding also too much overlap with the records of the earlier workers.

The Ovule and Female Gametophyte.

The young ovulate branches or strobilus units, unsuccessfully sought for in the resting winter buds, were first dissected out, still very minute, as the buds began to open in early April. They are carried, as usual, at the base of a vegetative branch. At this stage (Fig. 1), April 10th, there is yet no indication of the ovule proper. There are the two bracts, one of which carries the axillary structure which will eventually bear the ovule. Only very rarely was a corresponding structure found associated with the second bract, although in this figure a slight bulge on the bract at the right may indicate the position of such a structure which failed to develop. Later only two cases of paired ovules were met with. Little cellular differentiation except for the early procambium is shown, the whole being still meristematic.

Development is rapid. A week later (Fig. 2), April 17th, the axillary ovuliferous structure has increased in size and on the side away from its bract, its adaxial side, now shows a slight bulge—the position of the young ovule. Cellular differentiation has begun in the bracts which now show a well developed central procambium zone, an epidermis or protoderm dividing anticlinally, and a subepidermal zone in which vacuolation is distinct. Differentiation in the ovuliferous structure is still slight. It is not, however, proposed to follow in this paper the sequence of histological changes in these structures as they advance to maturity, even though such a sequence has not yet been described for any podocarp and is, in itself, of great anatomical interest especially in *Podocarpus*. But one point demands comment. A definite epidermal layer, dividing anticlinally, covers the ovuliferous structure except over the slight bulge of the incipient ovule. Apart from the bulging the ovule position is marked by the initiation, in the epidermis covering the bulge, of periclinal divisions which seem to be strictly limited to this zone. This is shown at a higher magnification in Fig. 3, which also indicates that in the centre of the ovular area is a small zone of cells which, though similar to the others, appear slightly larger. One cell here will shortly differentiate from its fellows as the single gynospore mother cell which becomes more deeply buried by the continued periclinal divisions of the outer layer and probably also of cells cut off from it. The regular arrangement of these cells increasing the outward growth of the nucellus is a striking feature practically up to pollination. The general form of the ovular structure in an older ovule (April 24th) is shown in Fig. 4. The integumental ring is now distinct, and the overarching growth above of the tissue which will ultimately form the so-called

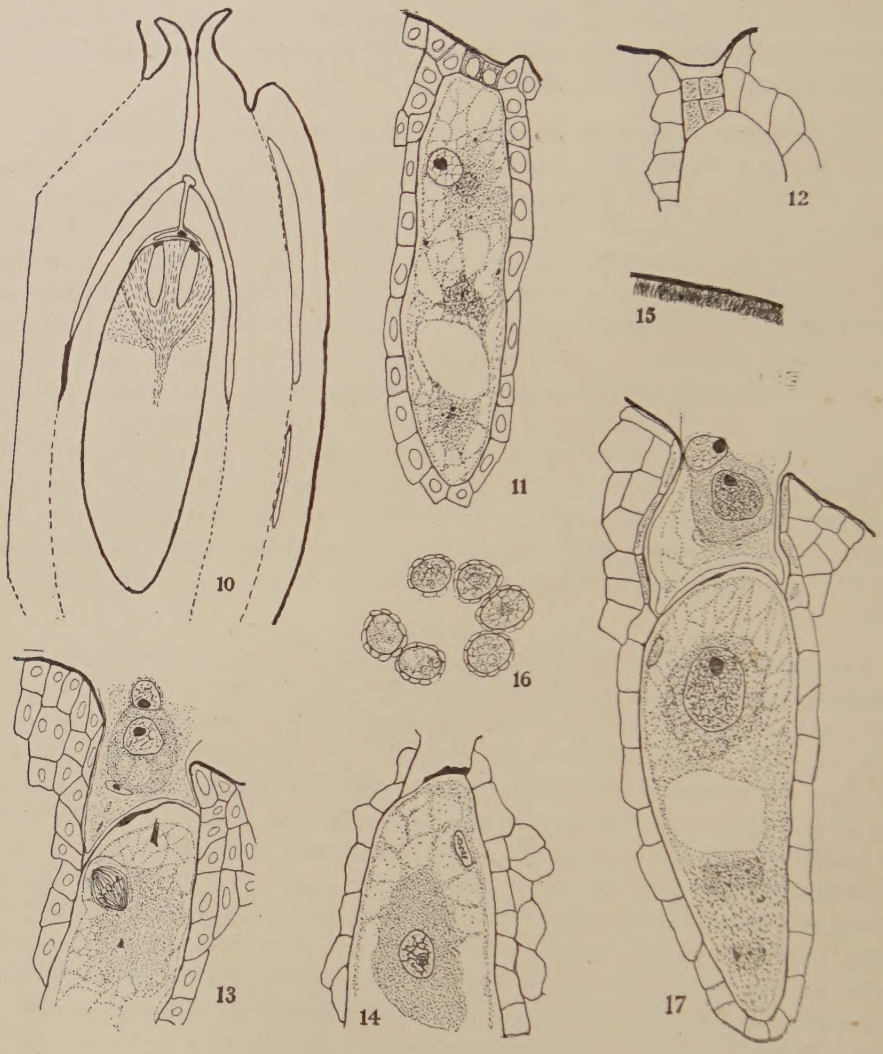
TEXT-FIGS. 1-9. **1**. Complete young strobilus with ovuliferous unit, *a*, axillary to bract, *b*. x 60. Apr. 10. **2**. Axillary unit later; *o*, ovule rudiment. x 80. Apr. 17. **3**. Detail of area, *o*, in **2** to show early periclinal divisions, *p*, delimiting ovule zone; anticlinal, *a*, elsewhere, x 250. Apr. 17. **4**. Ovule developing; *g*, gynospore m.c.; *i*, integument; *s*, scalar extension (epimatium). x 50. Apr. 24. **5**. Detail of ovular area of **4** showing continued periclinal activity. x 200. Apr. 24. **6**. Tapetum round binucleate gametophyte. x 250. May 21. **7**. Form of unit at pollination; diagrammatic to suggest histological differentiation; tapetum distinct; gametophyte already elliptical. x 28. June 16. **8**. Detail of one of the integument tips shaded in **7** to show poor closing cells, *c*, and the island of tracheide-like cells. x 260. June 16. **9**. General relation of parts later, especially narrow elliptical gametophyte just before alveolation; lengthening and thickening of micropilar integument, *i*; shaded area of **7** black. x 32. June 27.

epimatium has well begun. The cellular appearance at this stage is outlined, somewhat diagrammatically, in Fig. 5 which shows, as well as the gynospore mother cell, the periclinal divisions of the nucellus and the anticlinally dividing epidermis elsewhere. This short sequence of stages, as well as emphasising the histological questions arising, supports the obvious interpretation of the morphological nature of the strobilus unit, viz., the origin in the axil of a bract of a reduced fertile axis or telome system now bearing but one ovule.

The divisions of the single gynospore mother cell were not followed but they result in a linear row of three gynospores, of which normally the lowest functions. Sinnott (1913) records four gynospores in all the species of *Podocarpus* investigated by him, including *P. nivalis*, but states that three gynospores occasionally occur. It is not clear however in which species this latter appearance is found. Three have been also recorded by Looby and Doyle (1939, 1944) for both *P. andinus* and *Saxe-Gothaea*. The number of gynospores is thus presumably variable throughout the group, and is probably a point of little comparative importance. Sinnott (1913) also records the early development of tapetal tissue in his investigated species of *Eupodocarpus*. In the material of *P. nivalis* here examined, however, there is no definite tapetum to be seen until the functioning gynospore begins its growth, when this tissue develops rapidly (Figs. 6, 7) and remains a marked feature of the ovule till eroded away by the advancing gametophyte. Some tapetal development seems to be characteristic of the whole genus. Its extent and the stage of its first appearance are apparently variable and again are probably points of little comparative value.

The development of the female gametophyte is normal but presents certain interesting features of contrast to that described previously for the gametophytes in *P. andinus* and *Saxe-Gothaea*. The initial intense vacuolation of the gynospore before the first nuclear division, for instance, is a much less prominent feature, and from the beginning the gametophyte is elliptical (Fig. 7), rather narrowly so (Fig. 9) as it approaches the stage of alveolar formation. This stage as well as early cell formation and the appearance of archegonial initials proceeded in the normal coniferous manner, with the development of an upper " cone " area which is so prominent a feature in the other two types. In these, however, this " cone," when young, radiated upwards from a point which was approximately the centre of a sphere, so that the development of the *later* elliptical form of the gametophyte was accompanied by a rather intense upward meristematic development of the cone tissue. As in these two forms archegonial initials appeared early, this upward extension resulted in the very long narrow archegonia so characteristic of *Saxe-Gothaea* and many at least of the *Stachycarpus* species including *P. andinus*. In *P. nivalis* however the cone radiates when young from what is approximately the upper focus of a relatively narrow ellipse, the upward extension is much less, and the archegonia are relatively short. The proportions when the archegonia are still maturing are roughly indicated in outline in Fig. 10. which can be compared with Fig. 3 C in Looby and Doyle's paper (1939) on *Saxe-Gothaea* and Fig. 24, Pl. 7 of their paper (1944 a) on *P. andinus*. Later the rather compact appearance of the young cone cells is also shown by a central belt extending perhaps even below the

centre. It is down through this tissue that the young embryo cells will be later pushed by the elongation of the suspensors, but, while connected with the meristematically developed cone, it is almost certainly not directly derived from it.



TEXT-FIGS. 10-17. **10.** General relations, especially relative archegonium size. x 18. July 20. **11.** Typical young archegonium. x 185. July 20. **12.** One of the two cases of tiered neckcells. x 225. July 16. **13.** Ventral canal mitosis; male complex maturing; relations of pollen tube depression, archegonial membrane and degenerating neck cells. x 225. July 16. **14.** Egg nucleus, after v.c. division, retired to central cytoplasmic mass; v.c.n. to right. x 225. July 20. **15.** Gametophytic membrane. x 500. July 16. **16.** Central zone of gametophyte T.S. to show arrangement of six archegonia. x. 75. July 16. **17.** Archegonium and mature male complex just before initiation of fertilization stages. x 260. July 16.

A maturing archegonium, shortly before the ventral canal nucleus division, is shown in Fig. 11. This figure agrees very closely with the photograph of an archegonium of *P. totara* of approximately the same age given by Sinnott (1913), only differing in the slightly deeper and more lateral position of the nucleus. The three kinoplasmic masses and the large vacuole have also been described by Coker (1902) in the species examined by him. Coker, however, further describes and figures in this species great variation in the neck cell number which may range from one tier of two to more than 20 in several tiers derived by periclinal division. In contrast the present material of *P. nivalis* showed characteristically one tier of 5–6 neck cells. A doubling of the tier by periclinal division, as outlined in Fig. 12, was a very rare abnormality which was only found in two of the hundreds of archegonia examined. This contrast illustrates the difficulty of getting a clear picture of the condition of the neck cells throughout the genus, particularly with regard to the occurrence or frequency of periclinal divisions resulting in more than one tier. In the *Stachycarpus* species recorded by Sinnott (1913) and by Looby and Doyle (1944 a), and probably in all, there is one tier of from 8 to 16 cells. The latter workers have followed in *P. andinus* the regular division by anticlinal walls of the primary neck cell which gave this relatively large number all in one tier. So when Stiles (1912) merely records in his material of *P. macrophyllus* that there is great variation in neck cell number up to 18, but makes no statement on their arrangement, the tiering, if any, is left doubtful. Sinnott (1913) too, in his general statement covering the three forms, *P. totara*, its var. *Hallii*, and *P. nivalis*, simply states that the primary neck cell divides by periclinal and oblique walls into an irregular group of cells. Apart from the fact that it is not certain that the condition, so described as general, was in fact observed as actually occurring in *P. nivalis*, the statement, in the absence of figures, leaves the position obscure. Again, in the *Dacrycarpus* section Gibbs (1912) records one tier of 2–6 in *P. imbricatus*, while Sinnott (1913) for *P. dacrydioides* formally states that the primary neck cell divides first anticlinally and then periclinally to give a neck two or three tiers of cells in height. As these seem to be all the records available for *Podocarpus* it is clear that the condition in the main bulk of the species has still to be determined. It may well be that the occurrence, distribution, and frequency, of a tiered neck is a point of no comparative importance, the development of a tiered neck being just erratically irregular, but, since a single tier seems characteristic of all the other genera, a fuller range of data in *Podocarpus* is certainly desirable.

In all cases, however, in *P. nivalis* until disturbed by the pollen tube the necks are almost flush with the gametophyte surface or sunk only in a very shallow hollow. Their deep submergence, in the *Stachycarpus* group, due to the early upgrowth of the gametophytic tissue, is thus a point of very contrasting difference with the *Eupodocarpus* type. The condition in the *Dacrycarpus* section, judging from Sinnott's (1913) brief account of *P. dacrydioides* seems intermediate between the two extremes. The condition in the other sections does not seem to be recorded.

Shortly after the stage shown in Fig. 11 the division cutting off the ventral canal nucleus (Fig. 13) takes place. A range of stages was available—prophase, metaphase as here, anaphase, and late telophase. The mitosis appears to be

intranuclear, as a membrane can be seen round the mitosis up to the iniation of telophase. As the material was not suitably fixed for critical cytological analysis it cannot be formally stated that the visible membrane is a derivative of the original nuclear membrane, but such appears probable. The division in all cases seen was obliquely lateral, the resulting ventral canal nucleus being ordinarily found close to the archegonial wall and well down from the neck. The corresponding divisions recorded by Looby and Doyle for *P. andinus* (1944 a) were also oblique though higher up in the archegonium, but as these seem to be the only records in *Podocarpus* of the actual mitosis no general relationship can be suggested.

In preparation for the division the cytoplasm, particularly the upper kinoplasmic mass, becomes denser, and when the division is completed the functioning egg nucleus passes into this upper dense zone (Fig. 14). Here the nucleus rapidly increases in size, and shortly afterwards the archegonium is mature and ready for fertilization. A mature archegonium, with the pollen tube and male cell complex, is shown in Fig. 17, which gives at least a general idea of conditions at this stage. Occasionally the large vacuole may be replaced by cytoplasm with many small vacuoles, but more commonly the vacuole persists till after fertilization. This figure is included particularly for comparison with the corresponding stages in *P. andinus* (Looby and Doyle, 1944 a, Pl. 9, Figs, 42, 49) and *Saxe-Gothaea* (Looby and Doyle, 1939, Text-fig. 4 C and Pl. 2, Fig. 8). The pollen tube in *P. andinus* is a little slower in its development than in *P. nivalis* or *Saxe-Gothaea*.

The ventral canal nucleus commonly slowly degenerates but may persist till after fertilization.

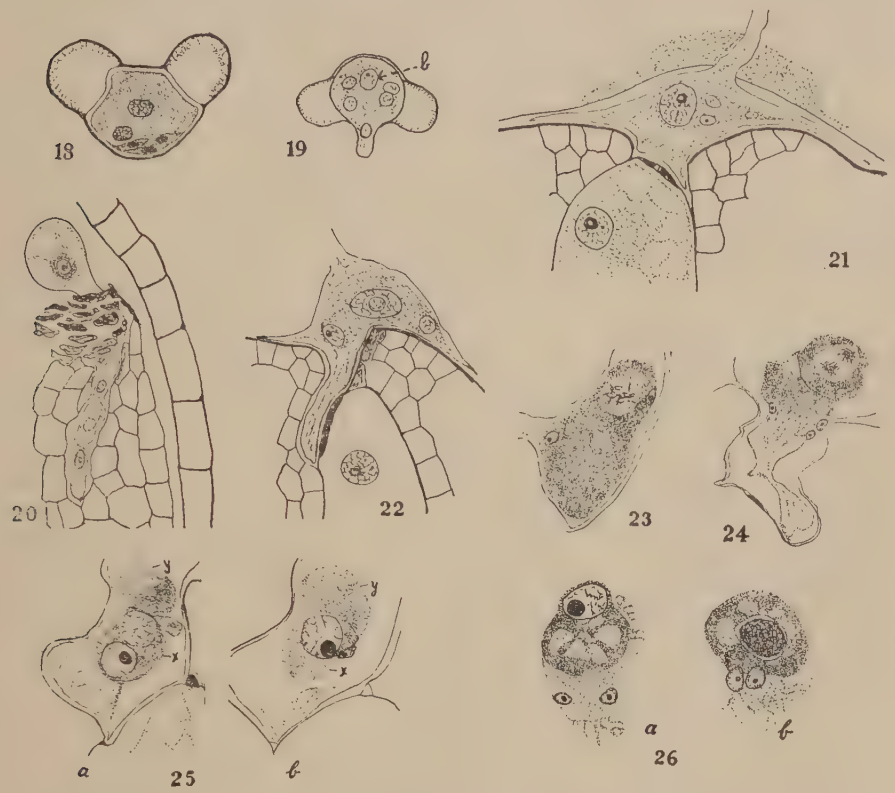
The Male Gametophyte.

Pollen, in the specimen of *P. nivalis* available, was ripe and shedding in the second and third weeks of June, during which period it was plentifully scattered over the ovulate strobili with successful results. It is received by an ovule which has attained the form outlined in Fig. 7, and by a pollination mechanism which, in its general features, resembles that in *Pinus* and which has been referred to elsewhere with that of *Podocarpus andinus*. (Doyle and O'Leary, 1935 b, Doyle, 1945).

In general the development of the male gametophyte agrees closely with that already described in detail for *P. andinus*, the agreement being so close as to suggest for this stage in the biocycle a great uniformity in the genus apart from the known small variability in the number of the prothallial cells. It agrees too with the rather scattered accounts of the male gametophyte given by the other workers frequently referred to above, and only a few points therefore of special interest, or of distinction from *P. andinus*, are referred to here.

A typical grain, when first seen in the micropile, shows (Fig. 18) three prothallial cells, the tube nucleus, and the generative nucleus, the latter usually a little off the middle line and frequently surprisingly inconspicuous. The only reference that could be found to the mature pollen of *P. nivalis* is a very brief statement by Burlingame (1908) that, at shedding, there are probably only the two basal prothallial cells as well as the other two nuclei. In the present material the prothallial

cells, in pollen shedding from the stamens as well as in early pollen in the micropile, are as usual very compact and tend to stain densely, so that the number of nuclei or cells could not always be determined with certainty, but in all cases in which the condition was clear, whether still in the pollen sacs or already in the micropile,



Text-figs. 18-26. **18.** Pollen at pollination. x 500. June 16. **19.** Pollen germinating; generative nucleus divided; body nucleus, *b*, distinct. x 400. June 27. **20.** Lagging of body cell in grain as tube penetrates nucellus. x 300. June 27. **21.** Tube "disc" splayed over gametophyte apex and beginning to burrow; young body-cell mass. x 260. July 12. **22.** Less common example of lateral burrowing. x 250. July. 12. **23.** Prophase of bodycell mitosis to one side of large cytoplasmic mass. x 260. July 12. **24.** Telophase of division; male cell cytoplasm differentiating from common mass; archegonial depression well formed. x 260. July 12. **25.** *a, b.* Just after the division; male nuclei already subequal in one male cytoplasmic mass, *x*. x 260. July 16. **26.** *a, b.* Oblique section of maturing male complex to show thinner zones in cytoplasm of functioning male cell; partial extrusion of non-functioning male nucleus; sterile nuclei. x 250. July 16.

three prothallial cells were seen, division apparently always taking place in the second. It is still quite uncertain whether prothallial cell number shows any systematic relations in podocarps, with the exception of *Pherosphaera* in which none are formed. Such a relation seems, on the scanty data available, rather unlikely, and pending the needed comprehensive survey this condition in *P. nivalis* is simply

recorded. The division to give body and stalk nuclei was not met with but must apparently take place shortly after the grains reach the micropile.

Germination is not very rapid but there is no marked resting period. The grains swell somewhat, the generative nucleus must divide, and the prothallial nuclei, freed from their membranes, come to lie loose in the cytoplasm getting larger and less dense. The stage most commonly met with about this period, Fig. 19, showed the tube well formed with the tube nucleus entering it, and, in the grain itself, four nuclei, one of which was slightly larger with a zone of associated cytoplasm—the young body cell. The other nuclei are clearly the three prothallial nuclei and the stalk nucleus, but the latter could not at this stage be distinguished from the others. As the tube penetrates the nucellus the body cell remains above in the grain for some time (Fig. 20). This lagging of the body cell seems to be quite a characteristic feature of podocarps in general. In those forms such as *Saxe-Gothaea, P. andinus,* and probably all species in the *Stachycarpus* section, as well as species of *Dacrydium,* in which the tube enters into a condition of winter rest after penetrating only a few cells deep into the nucellus, the body cell actually overwinters in the grain, and, in those more numerous cases in which the biocycle, as in *P. nivalis,* is completed in one year, the condition here figured parallels that in all the other genera in which any records of young male gametophytes are given or suggested in the literature. Collections were not at this stage sufficiently close to determine for what period the body cell delays in the grain. The delay can only be a matter of days but nevertheless the lagging is quite definite. It is perhaps of further interest that in a germinating grain a little younger than the stage in Fig. 20 the body cell is always accompanied by another nucleus when the others are already well down in the tube. This residual nucleus is almost certainly the stalk nucleus, but it too passes into the tube some little time before the first movement of the body cell. Elliot (1950) comments on the tendency in podocarps for the stalk nucleus to remain, practically up to fertilization, in close contact with the body cell. Such continued contact seems definite for *Pherosphaera* and *Saxe-Gothaea,* the stalk nucleus actually overwintering in the grain in the latter, the other nuclei having moved well down the long tube towards its tip. The tube in winter in *Podocarpus andinus,* however, is very short, and, though most of the prothallial nuclei have passed into it leaving the body cell behind, there are always a couple of nuclei in the grain. One of these is probably the stalk nucleus, but the nuclei are all very similar, and in later stages the distinguishing of the stalk nucleus from the others is not always certain. Elliot's point, however, is probably well chosen and deserves further careful consideration.

As a minor point it may be mentioned that, perhaps due to the density of the artificial pollination, the micropile often contained grains scattered along it which had not reached the nucellus. These grains, although in some cases at a relatively considerable distance from the nucellus, germinated to form rather narrow tubes which grew down the micropilar canal. Though germination was slower than in the case of grains actually on or very close to the nucellus yet some of these aerial tubes did reach the nucellus and penetrated for some distance. Pollination, however, was so successful that there was no possibility of determining whether such tubes, in the absence of actual nucellar grains, could bring about fertilization.

The functioning tubes grow through the nucellus fairly rapidly and then splay out like a thin disc over the top surface of the female gametophyte forcing and wedging their way in the narrow space between the gametophyte and the lower surface of the nucellar dome. This too, in some form, seems a characteristic behaviour in podocarps. Coker (1902) states for his investigated species that the tube has so splayed out before the archegonia initials were visible. In *P. nivalis* tube development is certainly more precocious than in *P. andinus*, comparing, of course, tube development from the initiation of active spring growth in both cases. There are too few data to venture an opinion whether such a difference is of sectional importance, but certainly the archegonia in *P. nivalis* are quite distinct, though far from mature, by the time the flattened disc spreads out over the gametophyte apex. As Coker, however, had very little pre-fertilization material, and as archegonial initials actually appear, probably in all species of *Podocarpus*, at a very early stage, it is thought that Coker's statement arose from a misinterpretation, very easy to make in any conifer if material is insufficient.

At any rate, when splayed out, some part of the flattened pollen tube end must make contact with the neck areas of the archegonia, which are practically flush with the surface of the female gametophyte. At this stage the material of *P. nivalis* clarified a difficulty earlier referred to in the account of *Saxe-Gothaea* (Looby and Doyle, 1939). It seems characteristic of podocarps approaching fertilization to find the tube with the male cells in an expanded chamber, well sunk in the gametophyte tissue and splayed out over the top of deeply sunken archegonia. This condition is shown, not only in *Podocarpus* but in *Dacrydium* (Stiles 1911), *Phyllocladus* (Young 1910), *Saxe-Gothaea* (Looby and Doyle, 1939) and *Pherosphaera* (Elliot, 1950). In *P. andinus*, where, as in other *Stachycarpus* species, the archegonia are already deeply sunken by surrounding upgrowth of gametophyte tissue when the pollen tube bursts through the nucellus, it has been shown (Looby and Doyle, 1944 a) how the chamber is formed by the burrowing of the tube down the canal so formed, and its expansion at the expense of surrounding cells. But here in *P. nivalis*, as in *Saxe-Gothaea*, the archegonia are still flush with the surface. Young (1910) had suggested for *Phyllocladus*, on very inadequate evidence, that the gametophyte tissue grew up round the pollen tube leaving the archegonia sunk at the bottom of the chamber so formed. Looby and Doyle (1939), however, presented evidence from *Saxe-Gothaea* which went to show that the pollen tube itself made the depression by actively separating the upper part of the archegonial wall membrance from the neighbouring cells, and forcing it down to form the bottom of the chamber. In *P. nivalis* it can be clearly seen that this is what actually occurs, and, since the end result is similar, it can be taken that this is essentially the behaviour in *Saxe-Gothaea* and *Phyllocladus* also. Thus Fig. 21 shows the young pollen disc initiating the activity. The specialized fungal-like burrowing of Fig. 22, though fairly frequent, is perhaps not so typical, but it emphasizes the nature of the early burrowing tip of Fig. 21. It is easy enough, without further detailed description, to follow how these early stages, as the tube burrows in all round the archegonial apex, pass on to those shown later, illustrated for instance in Figs. 13, 17, 24, 29. It is of special interest that Young's figures 27 and 28 for *Phyllocladus* show a remarkable similarity to figures 21 and 24 here given for *Podocarpus nivalis*. It is probable then that the

behaviour of the tube in *Phyllocladus* is similar to that here indicated, and that a similar activity occurs in all podocarps in which the archegonia are not embedded. The burrowing at initiation seems as a rule to be rather more active at one side. Fig. 22 is, of course, an extreme case, but in most cases at maturity the tube appears to have pressed down rather obliquely, and the freed archegonial membrane, on which the degenerating neck cells are as it were slung, commonly forms an oblique floor to the chamber. Nothing can be said concerning the nature of the action, and although a certain amount of breakdown commonly occurs in cells lateral to the tube in many cases no such necrosis can be seen.

In the meantime the body nucleus increases in size in preparation for division. It is associated with and surrounded by a large dense mass of cytoplasm at the edges of which the sterile nuclei are embedded. Only part of this, however, will form the actual cytoplasm of the male cell proper. This large cytoplasm mass increases also in volume and distinctness and takes up a position over the developing archegonial chamber, which is well advanced by the time the body cell nucleus is ready for division (Fig. 24). This division precedes the ventral canal division, and takes place while the body cell mass is either entering the cavity or just outside it. At early metaphase the mitosis can be seen at one side of the still undifferentiated cytoplasmic mass (Fig. 23), but by telophase it can be seen that round the mitosis a special zone of male cell cytoplasm is being differentiated (Fig. 24) from the rest of the mass in which lie the sterile nuclei. A somewhat similar carving out of functional male cell cytoplasm from a larger mass seems to occur also in *P. andinus* (Looby and Doyle, 1944 a) whereas in *Saxe-Gothaea* (Looby and Doyle, 1939) and *Pherosphaera* (Elliot, 1950) the original body cell cytoplasm seems to be wholly transformed in that of the gametes. Differences in gamete development therefore would seem to be another of those podocarpean points whose importance can be decided upon only after a further wider survey. By the time the two post-mitotic nuclei have returned to interphase the whole zone of cytoplasm and associated nuclei has moved well into the chamber, where maturation is completed. For some time after the mitosis the two nuclei are approximately similar in size and appearance, each nucleus showing rather thin nucleoplasm with a well developed nucleolus, as indicated by Figs. 25*a*, *b* from two rather obliquely cut sections on the same ribbon. Very rapidly the outer nucleus is partly extruded, and the inner enlarges, though both retain in general their original appearance (Fig. 13), the male cell cytoplasm being by now clearly differentiated. The whole complex *may* be surrounded by a thin membrane, but the fixation and staining used did not permit a decision on this point. The functioning nucleus (Figs. 17, 26*b*) soon becomes coarsely granular and reticulate, though the real cytological nature of this change could of course not be determined. The final condition just before fertilization is shown in general form in Fig. 17.

Before indicating the nature of the fertilization some further points call for brief comment. In *P. andinus* the non-functioning male nucleus was definitely enclosed in a narrow band of cytoplasm which was at least partly separated from the main bulk of the cytoplasm of the functioning gamete. It was possible to speak of two male *cells* though of very unequal size. In *P. nivalis* it is uncertain whether

such a special zone of cytoplasm, however narrow, encloses the non-functioning nucleus. The fixation and staining generally used were not suitable for the demonstration of delicate protoplasmic strands, though often enough the existence of a very narrow band of encircling cytoplasm was suggested. Some thin membrane, however, does most probably exist, as the non-functioning nucleus is not fully extruded and always enters the archegonium in the same relative position to the functioning gamete as it holds before entry. If no such membrane existed a separation of this second male nucleus might be expected in the turbulence of movement of actual entry. The condition is thus just a little more advanced than in *P. andinus*.

The general nature of the mature functioning male nucleus, the coarsely granular reticulum with the definite nucleolus, seems similar to that indicated, often very inadequately, in the different podocarps by other workers, but differs markedly from that described for *P. andinus* (Looby and Doyle, 1944 a), particularly in the nucleolus. In *P. nivalis* this is quite normally small in contrast to the enormous vesicular nucleus of *P. andinus*. So far the type of nucleolus described for this species does not seem to be recorded in any other podocarp, not even, to judge from Sinnott's (1913) illustrations, in the related species of *Stachycarpus* examined by him. However the only illustrations given by Sinnott of what he claims to be the male cell complex are his Fig. 2, Pl. 5 and his Fig. 50, Pl. 9. Both Looby and Doyle (1939) and Elliot (1950) have already commented on these, and agree that his Fig. 50 almost certainly shows a body cell with an associated sterile nucleus, probably the stalk nucleus (Elliot, 1950). Sinnot's Fig. 2, Pl., 5 is probably a young male complex, but the stage is inadequately drawn. The nature of the male nuclei in *Stachycarpus* species other than *P. andinus*, particularly in regard to the nucleolus, in thus still quite uncertain.

The very distinct zonation in the cytoplasm of the male cell described by Coker (1902) in his species does not appear in *P. nivalis*, and no reference can be found in the literature to such a zonation in the male cell of any other podocarp. Even Stiles (1912) in his account of *P. macrophyllus* makes no reference to such a zoning, and it will be remembered that the plant used by Coker was almost certainly a variety of *P. macrophyllus*. As Coker's material was mostly post-fertilization, and the only male cells present were apparently those in unsuccessful pollen tubes, the appearance may have been secondary due to ageing, but the point could only be cleared up by revision of Coker's actual material.

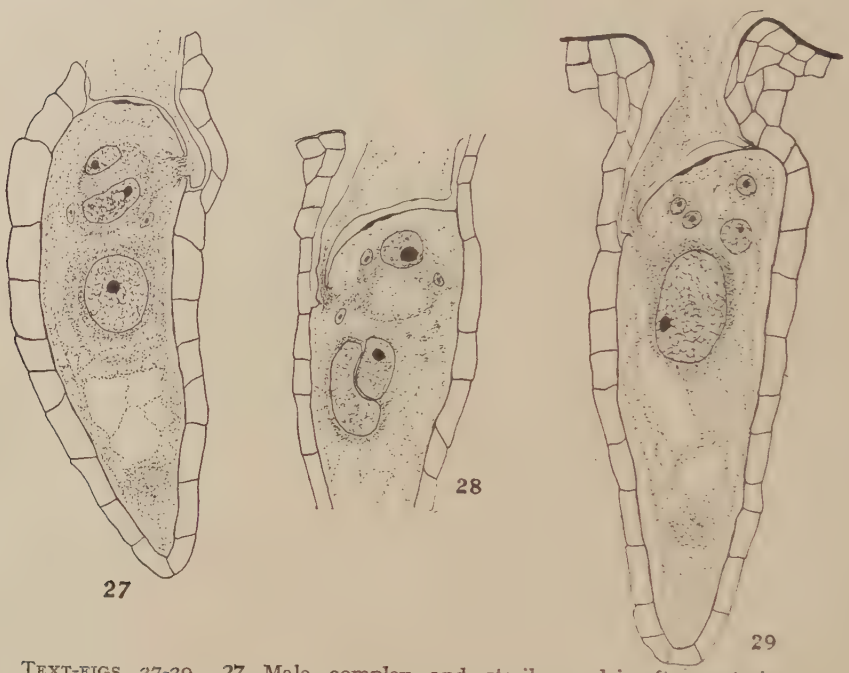
Figs. 26*a*, *b* illustrate a further point. These are from two successive sections and were chosen as, in sections so cut, there is shown more clearly the existence of a number of less dense zones in the cytoplasm of the male cell. Such zones seem quite characteristic. This appearance offers a possible explanation of an unusual male cell condition described and figured for *P. andinus* by Looby and Doyle (1944 *a*, Pl. 9, fig. 57, and p. 234). The less dense zone there described, which might be interpreted as a second subequal male nucleus but in which no nuclear structure was discernible in spite of its distinctness, is probably just the occurrence, unusual for *P. andinus*, of a single zone relatively larger but similar to those occurring in *P. nivalis*.

These short notes on selected aspects of male cell structure are here added to

call attention to the fact that such minor differences exist. Male cell structure is thus another aspect of the group which requires some further survey before it can be decided whether such variations have any critical value.

Fertilization.

The actual entry of the pollen tube was not observed, but, to judge from the appearance shown at subsequent stages, the tube does not enter between the neck cells but penetrates the archegonial membrane to one side. This membrane, with



Text-figs. 27-29. **27.** Male complex and sterile nuclei after entering archegonium laterally. x 225. July 15. **28.** Male and female nuclei in contact, male having slipped out of its cytoplasm; sterile nuclei and second male present. x. 225. July 15. **29.** Fusion complete; male cytoplasm dissolved but second male and sterile still distinct. x 260. July 15.

the collapsed neck cells associated roughly centrally, stretches as already described, somewhat obliquely across the floor of the chamber formed by the burrowing and expansion of the pollen tube. The latter seems to press the membrane down still lower just previous to fertilization, then probes yet further down one side and enters by penetrating the membrane at a lateral point (Figs. 27, 28, 29). The membrane does not seem to be coarsely ruptured but definitely penetrated with a narrow orifice. Naturally for accurate detail on this point preparations would be necessary in which the actual entry had been secured, but it seems the obvious interpretation to put on the appearance shown, particularly by the residual pollen tube cytoplasm, at the zone of entry outlined in Fig. 28. This method of entry

is also very different from the clean entry through the necks, and the associated phenomena, shown by *P. andinus*. How far either are characteristic of the genus, or whether still other methods of entry are used, is quite uncertain, as there seem to be no other proper records of pollen tube entry in *Podocarpus*. Thus Sinnott (1913) simply says for the *Stachycarpus* species that fertilization was not observed, and for *P. dacrydioides*, representing the *Dacrycarpus* section, that details of fertilization resembled those in *Eupodocarpus*. Here, in his generalized account of *P. totara*, its var. *Hallii*, and *P. nivalis*, all three of which he suggests are in very close agreement and so can be covered by the one generalized description, he says merely that one branch of the pollen tube enters and breaks through the neck of the archegonium. A close agreement, on systematic grounds alone, would be expected between *P. nivalis* and *P. totara*, but clearly Sinnott's record clashes with the account here given for *P. nivalis*. It may be that the entry stages were not actually available to Sinnott for this species, and that the remark about entry through the neck was made on the general grounds that entry of a pollen tube into an archegonium is to be expected through the necks. He gives, too, a drawing (his Pl. 5, fig. 1) of an archegonium of *P. totara*, shortly after fertilization with the male and female nuclei in contact, in which there is no indication of a chamber gouged out by the pollen tube and having for its floor the separated archegonial wall membrane. A short narrow tube puts the narrow top of the archegonium in contact with the exterior. At a later stage like this it is perhaps possible, but very unlikely, that the appearance described by Sinnott is due to precocious post-fertilization collapse. It is more likely that, in spite of Young's (1910) previous account of the sinking of the pollen tube in *Phyllocladus* and of Stiles' record (1911) of a similar feature in *Dacrydium*, the conditions about this stage in *Podocarpus* were not appreciated by him. Clearly therefore with data available only for *P. andinus* and *P. nivalis*, and these so different, the position is unsatisfactory.

Most of the contents of the tube are expelled into the archegonium. The conditions immediately after entry are outlined in Fig. 27, which shows the lateral position of pollen tube entry and, within the archegonium, the whole male cell complex and some sterile nuclei. Fig. 28 shows that the functional male nucleus slips out of its cytoplasm impinging against and indenting the female nucleus, and that the second male nucleus and the sterile nuclei are still visible. Fusion of the nuclei then ensues. Several stages of partial fusion were available, but as the cytology of the process is quite obscure only the final stage is outlined in Fig. 29. These figures, illustrating stages about the fertilization period, are perhaps somewhat diagrammatic, but comparison of them shows that the male cell cytoplasm is simply assimilated in the general egg cytoplasm, any denser cytoplasm round the female nucleus being merely the denser zone of archegonial cytoplasm earlier formed. The general archegonial cytoplasm tends to become somewhat more uniform, sometimes, by the obliteration of the large vacuole, quite uniform, but more commonly this vacuole is still retained. The behaviour of the second male nucleus and the sterile nuclei varies. Occasionally they gradually disintegrate, but more often they remain quite distinct or even increase in size and definiteness, though never seen, at least in this material, to divide. When these nuclei, however, persisted well into the free nuclear stages of proembryo development, as they occasionally

did, they certainly added a little to the difficulty of a sure interpretation of these stages.

It is proposed to deal in the second communication of this series with the embryogeny which is now initiated.

Additional Data and Remarks.

Certain aspects of structure or comparison were omitted from the general account so that that account would give a continued history of gametophytic development not too much interrupted by special descriptions. These are considered in this section, and are mostly considerations that naturally arise in any comparison of *P. nivalis* with *P. andinus*.

(A) Micropile Closure.—Ovular form, shortly after pollination, has already been outlined in Fig. 7. The various zones, nucellar, integumental, epimatial or scalar with its differentiating vascular tissue, have each a very characteristic histological structure and would each deserve a detailed description for comparison with those of other species. One point, however, seems to claim attention here. Fig. 8 shows the detail of the area stippled in Fig. 7 and illustrates what must be a very widespread feature in species of *Podocarpus*, viz. the zone of cells with strong spiral thickening. At this stage they are still living, as indicated by the nuclei, but later they lose their contents and look like a mass of tracheidal cells, though no special bordered pits could be seen in their walls. In Gibbs' (1912) broad survey of histology and development in *Podocarpus* this island of tissue is mentioned and, without any real detail, is merely reported as occurring in all the species examined, i.e., 2 of *Dacrycarpus*, 2 of *Stachycarpus*, 1 of *Nageia*, and 8 of *Eupodocarpus* including *P. nivalis*. Its occurrence in *P. salignus*, as well as in *P. nivalis*, can be confirmed from personal observation, and there is no reason to doubt its occurrence in the other species mentioned, though only one rather inadequate figure from an unnamed species is given in illustration. But it is not universal in the genus as it definitely does not occur in *P. andinus*, in spite of Gibbs' assumed record for *P. ferrugineus* and *P. spicatus*, nor could it be seen in available local material of *P. latifolius*. It may, however, be present in small amount in this species, incidentally not on Gibbs' list, as the local available material was perhaps a little too old. The histological detail of this tissue, its exact location, and its amount, would seem to vary, like all other histological detail in podocarps, from species to species, as the detail was quite distinctly different in the two species here available in quantity viz. *P. nivalis* and *P. salignus*.

As shown in Fig 8 it seems to be located in relation to the closing cells, the detail of which must also vary in the genus, as the condition here outlined for *P. nivalis* is decidedly different from that previously described for *P. andinus*. The term closing cell is here used to designate those cells of the inner epidermis of the upper micropile which elongate inwards with or without division, and, so defined, it is probable that in all species of *Podocarpus* they are of relatively less importance than in many other conifers. Here in *P. nivalis* they are particularly poorly developed, being limited to a very restricted zone and showing little further increase in size and number over that indicated in Fig. 8. But even when much better

developed, as in *P. andinus*, they still play only a minor part in micropilar development after pollination. After pollination in *Podocarpus* the micropilar part of the integument, *i.e.* the part above the level of the nucellar apex, increases markedly in length as the ovule grows, and the proximal part, by general tissue increase but not by special closing cells, thickens considerably, forming a long narrow canal from the exterior to the small chamber over the nucellus upon which by this stage the pollen would normally be developing. Comparison of Figs. 3, 5, 10, Pl. 6. in Looby and Doyle's (1944 a) account of *P. andinus* shows very clearly the conditions for this species in which can also be seen the manner in which the canal is extended by cellular increase in the adjoining scalar tissue. Comparison of the diagrammatic Figs. 7, 9, 10 here given for *P. nivalis* indicates a similar integumental development, although in this species a scalar development beyond the micropile proper is much less. The small black area in Fig. 9. indicates the area stippled in Fig. 7 and brings out the relatively minor importance of the true closing cells.

(B) THE GAMETOPHYTIC MEMBRANE.—The membrane which separates the developing gametophyte from the surrounding nucellar mass is a thick two-layered membrane, (Fig. 15), which seems to resemble rather closely the membranes in *P. andinus* and *Saxe-Gothaea* (Looby and Doyle, 1944 a, Pl. 7, fig. 25 ; 1939, text-fig. 3 C). It increases in thickness as the gametophyte matures, and is fully persistent up to an advanced stage of embryogeny, the latest stage reported in this study. It thins out over the archegonial apex, but not markedly. Such a membrane of varying thickness, is of course characteristic of most conifers and is commonly called the megaspore membrane. In the conifer studies, however, of which this paper is a part, it has been referred to as the gynospore membrane. In view of the obvious fact that it is a thin membrane, often demonstrable with difficulty at the gynospore stage even in those cases in which it is thick and firm at maturity, and also that normally in such cases it develops *pari passu* with the gametophyte, it seems plausible to suggest that it has some function in relation to the gametophyte, and is not merely a static vestigial morphological descendant from the megaspore membrane of a mythical condition of heterospory. It is thought that this viewpoint may best be emphasised by calling it the gametophytic membrane.

Such a membrane, though very variable in thickness, has been described in some representative of each of the podocarpean genera. Detailed references are available in Schnarf's (1933) monograph. In *Dacrydium*, however, there seems to be a record for only one species, *D. laxifolium* (Thomson, 1905). It is remarkable that Sinnott (1913), though giving some details of the gametophytes of four species of *Dacrydium*, omits any reference to the nature of the membrane in them in spite of the fact that he carefully recorded it for all the species of *Podocarpus* which he investigated. The condition in *Dacrydium* as a whole is thus uncertain. In the genus *Podocarpus*, with which this account is more closely concerned, there are records by Gibbs (1912), Stiles (1912), and Sinnott (1913), as well as personal records, for the presence of at least reasonably defined membranes for 3 species of *Stachycarpus*, 2 of *Dacrycarpus*, 2 of *Nageia*, and 10 of *Eupodocarpus*. This wide distribution, therefore, renders rather remarkable the fact that Thomson (1905), in his systematic study of the membrane in all the gymnosperms, was unable to detect

any such membrane either in his own material of *P. macrophyllus* var. *Maki* or in the material already worked over by Coker (1902), which it will be remembered was probably actually the same variety. Stiles, too, records for his material of *P. macrophyllus*, collected in Tokyo and recorded apparently as the basal species, that the membrane though present was very thin. Thomson and Coker's type, being a garden variety, may of course not be quite normal, and Stiles' material may have been from the variety also. Some little doubt may be entertained in any event concerning Stiles' record, since Sinnott records a good membrane for all the six species of *Eupodocarpus* examined, including *P. elatus*, a species whose membrane is described by Stiles as " very poorly developed." Were it not for these uncertain records the existence of a good two-layered membrane, such as exists in *P. nivalis*, could be accepted as a character in *Eupodocarpus*, and in this respect this latter section could be considered to have shown no change from the more primitive type. Stiles' further record of a very thin membrane in *P. nagi*, would thus also need revision, although such a sectional modification would be quite reasonable in view of Sinnott's record that the membrane in *P. dacrydiodides*, though quite distinct, is a little thinner than that in the *Eupodocarpus* section. The family as a whole seems to yield the following picture. A well developed membrane is shown by *Dacrydium* (at least some species), *Saxe-Gothaea*, and *Phyllocladus*. In *Pherosphaera*, a genus so specialized that its inclusion in the family was often questioned, the membrane is definitely (Lawson, 1923, Elliot, 1950) very thin. Such a change might reasonably be expected. Basally in *Podocarpus* the membrane is also well developed, a condition continued in the advanced *Eupodocarpus* section and in the *Dacrycarpus* section, though a thinning may have supervened in the section *Nageia*. Clearly the disappearance of the membrane, or its extreme tenuity, in occasional species of *Eupodocarpus* related to other species with a characteristically thick membrane does not seem to fit the picture. Confirmation or correction of these latter records is thus obviously needed.

(C) Archegonial Form.—Reference has earlier been made to the contrast between the relatively short archegonia with bluntish ends in *P. nivalis* and the long pointed archegonia of *P. andinus*. This long form is also shared by other species of *Stachycarpus*, certainly *P. spicatus* and *P. ferrugineus*, by *Saxe-Gothaea*, and by at least some species of *Dacrydium* such as *D. Colensoi* (Stiles, 1911). It is suggested that *Saxe-Gothaea* might be taken to represent the basal type, though in this latter the archegonia need not have been quite so long and pointed. From this there seems to be shown in podocarps a general tendency towards archegonia of the *P. nivalis* type. This is clear in the *Eupodocarpus* section, but even in some of the advanced species in the *Stachycarpus* section, such as *P. amarus* or *P. usumbarensis*, the archegonia, according to Bucholz (1941), are already somewhat shorter and blunter than in *P. andinus* or *P. spicatus*. In *Dacrydium* the same tendency seems to appear if Sinnott's (1913) fig. 48, Pl. 9 of *D. cupressinum* be compared with Stiles' (1911) account of *D. Colensoi*. It is not easy to be certain from any of the accounts about conditions in *Phyllocladus*, but it seems to take a median position. *Pherosphaera* (Elliot, 1950) shows, as might be expected, a very advanced condition of this tendency, which is also expressed in the reported complex of *Microcachrys*. Shorter blunter archegonia thus appear in general to be associated with advance,

and long pointed ones with a retention of a primitive gametophyte stage. It is of interest perhaps to recall in this connection the long narrow archegonia of *Cephalotaxus*.

(D) ARCHEGONIAL EMBEDDING.—Reference has also been made to the fact that while in *P. andinus* the archegonia are early embedded by upgrowth of gametophytic tissue, the archegonia in *P. nivalis* are practically flush with the surface till pressed down by the attacking pollen tube. This raises a problem. In *Podocarpus* this deep embedding seems characteristic of typical *Stachycarpus* species, it is shown, though perhaps to a lesser extent, in the *Dacrycarpus* section, but does not occur in *Eupodocarpus*. A sequence seems suggested from an embedded to a flush or shallow position in this genus. But definitely in *Saxe-Gothaea*, in spite of their otherwise close resemblance to those of *P. andinus*, the archegonia are flush or only sunk in a very shallow pit when pollen tube attack begins. They are flush too in *Phyllocladus*, and, allowing for the development of the archegonial complex, in *Microcachrys* also. The records for *Pherosphaera* are, however, remarkably conflicting. Lawson (1923 b) definitely states that the archegonia are but slightly sunken in a shallow pit, and figures one such (fig. 20) with a pollen tube, containing a well developed body cell, in contact with the necks. Elliot (1950), on the other hand, records that they are well buried, and that the tube must bore through prothallial tissue to reach them. The position in *Dacrydium* too is a little uncertain. Sinnott (1931, Pl. 9, fig. 48) gives a photograph of two archegonia in *D. cupressinum* which, though not mature, are obviously well advanced. At such a stage in *P. andinus* the necks would be already deeply embedded, but here in *Dacrydium* they are still flush. It would seem unlikely that anything more than a shallow pit could be formed before pollen tube activity began, yet in his description in the text of development in *Dacrydium* he says that "the prothallial tissue often grows up over the archegonia." This description, however, is a generalized one covering not only *D. cupressinum* but also *D. Bidwillii*. He gives a drawing (Pl. 5, fig. 3) showing in this species, an archegonium, but with no associated pollen tube, which is definitely embedded though less deeply than in *P. andinus*. The statement quoted therefore may not necessarily refer to *D. cupressinum* but to *D. Bidwillii*, in which case the reference is actually to a type which is a stachycarpean species or a near relative (Sahni and Mitra, 1927). Alternatively, in almost all cases archegonia not reached by a pollen tube may with age, become embedded, perhaps only slightly, perhaps more deeply, and this could also happen in *D. cupressinum*. As no other evidence seems to be available the position is left unsatisfactory in the extreme, but on the basis of Sinnott's *photograph* of the archegonia of *D. cupressinum* it is suggested that, provisionally, *Dacrydium* may also be put with *Saxe-Gothaea*, *Phyllocladus*, *Eupodocarpus*, and *Microcachrys*, as having archegonia still in a shallow position when reached by a pollen tube. The extreme condition in *Stachycarpus* would thus appear rather exceptional in the family.

On such data no strong statement can be made, but a suggestion may be ventured whose value would be greatly strengthened if species of *Dacrydium*, particularly those which have a two-year biocycle, should actually prove to have shallow archegonia as here provisionally accepted. Shallow archegonia, then, as

in *Saxe-Gothaea*, would be basal in the family and sunken secondary. In *Pherosphaera* for instance the sunken condition, if such be confirmed, would be just one of the specialized divergencies in this very specialized genus. In *Podocarpus*, however, a sunken condition basal to the genus, as might appear from the cursory consideration of the genus above, would mean that a change from the typical shallow to the sunken condition must have taken place in the primitive forms, now represented by the stachycarpean species. This condition would have been gradually lost in the advance to *Eupodocarpus*, in which section the shallow condition would have been again secondarily acquired. This view rather assumes a direct step by step gradation from *Stachycarpus* to *Eupodocarpus*, which is not the most likely evolutionary plan in a group which is probably very old, and in which the living forms are probably but the end survivors of many diverging lines. It is more likely perhaps that *Stachycarpus* represents a side branch from the main evolutionary line of the genus, a branch given off quite early and so retaining many primitive features but developing some special features of its own including the sinking of the archegonia. The main line, culminating in the large section of *Eupodocarpus*, could well have retained the primitive shallow condition which would thus still be primary in, say *P. nivalis*, as it seems to be in *Saxe-Gothaea*.

The point here dealt with may appear of little importance, but it illustrates the difficulty of comparative treatment of the podocarps as a whole, arising from lack of data. As far as the data go, however, they seem to support those in (*C*) in assigning a basal primitive condition to the *Saxe-Gothaea* type.

(E) Archegonial Number.—In the material of *P. nivalis* here examined the number of archegonia commonly ranged from 5 to 7, in some cases rising to 9. This is somewhat in excess of Sinnott's (1913) record of 3 to 6 in his generalized account of *Eupodocarpus* species, which included *P. nivalis*, but in no case observed in this study did the number fall below 5. The records for other species of *Podocarpus*, excluding *Stachycarpus* species, on the whole range higher, *i.e.* 5–10 for *P. macrophyllus* and *P. latifolius* (Coker, 1902 ; Stiles, 1912), one record by Stiles of 14 for *P. latifolius* confirmed by personal observation of Irish grown material, 5–12 for *P. dacrydioides* (Sinnott, 1913), and up to 19 for *P. nagi* (Stiles, 1912). In contrast, 2, or only occasionally 3, seem characteristic of the *Stachycarpus* species definitely recorded, viz. *P. andinus*, *P. spicatus*, and *P. ferrugineus* (Sinnott, 1913 ; Looby and Doyle, 1944 a). This is of course a poor record from the many species of *Podocarpus*, but there would appear to be recognizable within the genus this other tendency towards an increase in archegonial number. This tendency, however, seems to be limited within the family, with the possible exception of *Microcachrys*, to *Podocarpus*, the number recorded for the other genera being always small, thus *Acmopyle* has probably only 1 (Sahni, 1920), *Dacrydium* 2–3 (Stiles, 1911 ; Sinnott, 1913), *Saxe-Gothaea* 2–3 (Looby and Doyle, 1939), *Phyllocladus* 2–3 (Young, 1910 ; Holloway, 1939), *Pherosphaera* 3 (Lawson, 1923 a), though recently Elliot (1950), while agreeing that 3–4 is most common, has recorded up to 6 occasionally in this genus. Apart from this apparent slight expression of the tendency to number increase in *Pherosphaera* 2–3 archegonia seem basally characteristic of the family with a very definite tendency to number increase in *Podocarpus* coupled apparently

in this genus also with the general tendency to shortening. In this feature too it would seem that *Saxe-Gothaea* could be taken as representative of the bsaal condition.

Microcachrys, on our present knowledge, seems exceptional. According to Lawson (1923 b) the number of archegonia would be a little high, 5–6, but he describes them as arranged in one cupressinean-like complex, fertilized incidentally from pollen tubes each with two equal and rather cupressinean-like male cells. This record still needs rechecking, especially in view of the fact that Thomson (1909) had earlier recorded for the genus, but at a younger stage, a number, unspecified, of widely scattered archegonial initials. However an increase in number and a condensation to a complex may well have developed in the genus. Most of the workers on *Podocarpus* referred to above record that in species with the higher numbers the archegonia may be close, two sharing laterally the same jacket layer, and also, though rarely, that two or more archegonia may be enclosed in a common jacket, phenomena shown also by the present material of *P. nivalis*. The actual final stage simply depends on the relative closeness of the initials. A complex could clearly have arisen readily enough, although, from a consideration of the arrangement of archegonia in Fig. 16 a condition would rather be expected like that in *Cunninghamia* (Miyake, 1911) where the archegonia form a ring enclosed in a common jacket and surrounding a mass of sterile tissue. As shown in Miyake's Figs. 69 and 70 a tangential section of such an arrangement can give the appearance of a compact complex. Certainly also in *P. latifolius* the 14 archegonia are closely arranged in a ring round a central zone of sterile tissue, with several in lateral contact and at places as many as three in a common jacket.

In connection with both number and form of the archegonia it is interesting to compare, say, *Cephalotaxus* with 3, or a maximum of 4, longish pointed archegonia each with its jacket, or the broad similarity with the Pinaceae. Here the archegonia are mostly large, few, and separate, and show in some cases such as in *Pseudotsuga* a surprising resemblance to *Cephalotaxus*. Such comparisons cannot be pressed too hard, nor is this the place to enter into a full survey of conifers and taxads, but they support the view that, in podocarps, the *Saxe-Gothaea* type is basal in number and in form of the archegonia.

(F) THE MALE GAMETOPHYTE.—The male gametophyte of *P. nivalis* is quite characteristic of most of the family in the winged grains, the multiplication of the original prothallial cells, and the extreme inequality of the gametes. Pollination mechanisms have been discussed elsewhere (Doyle, 1945), and it is reasonably certain that the winged condition of the pollen grain is basal in the family, so that the wingless condition in *Saxe-Gothaea*, for instance, is associated with a specialized pollen and ovule behaviour and is secondary. Two prothallial cells, which may increase by division, are clearly basal also, and the unique absence of these cells from the grain of *Pherosphaera* is certainly secondary. There may be differences in body cells, or male cells, or the development of either, but these differences are mostly minor and have been sufficiently commented upon earlier in this account. In general the uniformity in the nature of the male cell complex is remarkable. Even so specialized a genus as *Pherosphaera*, whose very small female gametophyte with relatively minute archegonia often so oblique as to be almost lateral is so

different from that of *P. andinus*, the male cell complex (Elliot, 1950, text-fig. 7) could be that of any species of *Podocarpus*. This condition of extreme inequality of the male cells is clearly derivative, but here again *Saxe-Gothaea* may be taken as more representative of the basal type, showing as it does equal or almost equal male cells. From some such condition, retained also perhaps in *Microcachrys*, the condition of extreme inequality must have been derived. The similarity of the male cell complex in *Podocarpus*, *Dacrydium*, *Phyllocladus*, and *Pherosphaera*, need not demand a close common origin for these genera, but rather suggests, as a peculiarity of podocarpean relationship, a general tendency towards the suppression of one gamete, a tendency independently expressed in different and even strongly diverging lines. Such a tendency is also seen expressed in the taxads and even in the very different and distant *Sciadopitys*, in which the male cell complex is extraordinarily podocarpean in its structure (Tahara, 1940).

(G) Fusing Nuclei at Fertilization.—It is not always possible to give a definite and accurate measure of the relative sizes of the male and female nuclei, as both may change rapidly, often at different relative rates, during the period from the first penetration of the pollen tube into the archegonium to the stage of actual contact. The stage of actual contact of these nuclei in the archegonium is a definite stage conveniently specified as the *fertilization* stage. It is comparison between different types at this fertilization stage which is most definite and therefore possibly most useful. Comparisons at stages before pollen tube entry may only have an approximate value unless the progress of the process is clear from a close series of preparations, as in a very limited series the male cells or the archegonia or both may appear to be mature when in fact they are not, and in such cases there may be only a rough idea as to how much further development must proceed before fertilization is actually effected.

A full critical survey of this male to female nuclear ratio has not yet been made, but in general the values of this ratio seem, provisionally and approximately, to be family characteristics—a gross inequality in Pinaceae, equality in Araucariaceae, a marked but not gross inequality in taxads and *Cephalotaxus*, as examples. A survey of this feature in the *Podocarpaceae* would then be of interest even though such a survey reveals the usual unsatisfactory position in the family owing to the absence in too many critical cases of figures or descriptions of suitable stages.

In *Podocarpus nivalis*, Fig. 28, the egg nucleus at fertilization is roughly 2 to 3 times the size of the male. This is another striking contrast with *P. andinus* in which the female nucleus is strikingly larger than the male, showing what may be called gross inequality (Looby and Doyle, 1944 *b*, Pl. 11, figs, 4, 5). There is here within the one genus a much greater difference than occurs even in the full range within any other family, and so the *Podocarpaceae* would seem to demand special attention.

The other available data on *Podocarpus* may first be considered. Tahara (1941, text-fig. 6) illustrates fertilization in *P. nagi*, of the *Nageia* section, and here the ratio clearly lies between these extremes, the species showing a marked but not gross inequality. Otherwise in *Podocarpus* the only available records, mostly inconclusive, are from the generalized statements of Sinnott (1913). Thus for the

species of *Eupodocarpus* investigated by him he states that the male nucleus at fertilization is about one half the size of the egg nucleus, which agrees with the present record for *P. nivalis*. He figures, however, the nuclei in contact (Pl. 5, fig. 1) in *P. totara*. The male nucleus in this figure is represented by a narrow crescent closely pressed to the egg nucleus, and the total volume of the male *so drawn* is quite a small fraction of the volume of the female. This could be, and probably is, the outline of a section in which but an edge of the male nucleus is cut, its main bulk lying in another plane, but also it could be a relatively small nucleus flattened in the course of the fusion process. Thus although the factual conditions in *P. nivalis* confirm his textual statement there is left an element of doubt as to whether in *Eupodocarpus* some species may show a nuclear difference amounting almost to gross inequality. Stiles, however, gives a figure (1912, Pl. 48, Fig. 31) for *P. macrophyllus* before pollen tube entry but with the male complex well formed. In this the relative proportions are much as in *P. nivalis* at the same stage, and it is safe enough to assume that the ratio would be, at least roughly, of the same order also at actual fertilization. If Sinnott had drawn no figure a condition as in *P. nivalis* would have been accepted without question as characteristic of the *Eupodocarpus* section. It may be that an undue importance is being given to what may have been meant to be merely an illustration of a general nature, but an uncertainty is introduced which only further investigation can clarify.

In the *Stachycarpus* group the details of fertilization were not observed, but Sinnott states again, and it is this which adds to the uncertainty in *Eupodocarpus*, that just previous to fertilization the functional male is about one half the size of the female. In fact, however, he figures the apex of an archegonium of *P. spicatus* showing a very large female nucleus which is suggestive of that in *P. andinus* and which is relatively enormous in comparison with the nucleus in a corresponding figure for *P. totara* (Pl. 8, fig. 41 and Pl. 7, fig. 29). A male nucleus one half the size of the female one figured for *P. spicatus* would be so huge for a male cell as to be quite unlikely. He further figures for *P. ferrugineus* (pl. 5, fig. 2) the condition just before pollen tube entry showing the male cell complex in the burrowed archegonial chamber above what is clearly a fairly mature archegonium whose nucleus is very large. Here without question the relative nuclear size at fertilization must closely agree with that in *P. andinus*. So in spite of Sinnott's general statement it must, it seems, be taken that the *Stachycarpus* species, as so far investigated, are characterized by a gross inequality of the fusing nuclei. A knowledge of conditions in the higher stachycarpean forms such as *P. amarus* would be of great interest.

An equally definite statement, however, is not possible for the *Dacrycarpus* section. Sinnott states again for *P. dacrydioides* that the details of fertilization agree almost entirely with *Eupodocarpus*, which would mean an egg nucleus about twice the size of the male nucleus. He gives a photograph (Pl. 8, fig. 37) of the nuclei in contact in an archegonium in this species which certainly agrees with his published *figure* for *P. totara*, as it shows quite a small male in contact with a relatively large female nucleus, but does not agree with his *statement* for *Eupodocarpus* that the female is only about twice the male. This figure, which *looks* as if the two

nuclei were cut medianly, is a somewhat advanced stage of fusion and rather corresponds with Looby and Doyle's (1944 b) figure (Pl. 11, fig. 7) of a similar stage in *P. andinus*. If Sinnott's figure is from a median section there is the remarkable result that, both in absolute and in relative size, the nuclei in the two figures are of the same order, an order which makes the female about 30 times the male. Actually in *P. dacrydioides* the female nucleus is a little smaller than in *P. andinus*, and, relatively, the male appears to be a trifle smaller still, but the differences are insignificant and might easily be reversed in other cases if a wide range of material were available. On this figure of Sinnott's from *P. dacrydioides* a gross inequality of the nuclei must be taken for the *Dacrycarpus* section unless the figure be rejected, and such a clear photograph as this could not be rejected unless a re-examination of the material showed that the male nucleus had, in the section photographed, merely been cut at its edge.

The situation in *Podocarpus* is thus singularly unsatisfactory, and is made all the more unsatisfactory by a consideration of *Pherosphaera*, *Saxegothaea*, and *Phyliocladus*. *Pherosphaera* (Elliot, 1950, Pl. 10, figs, 16, 17), rather surprisingly in view of the small size of gametophyte and archegonia, has a relatively large egg nucleus and a ratio of female to male perhaps best described as one of marked but not gross inequality. *Phyllocladus* (Young, 1910, fig. 35) shows a ratio of the same order though perhaps a little nearer to that in *P. nivalis*. Comparison of Young's figures 32 and 35 is a good example of the point made at the beginning of this section. Fig. 32, described as a mature stage, is a typical stage before pollen tube entry, when the male cells are apparently ripe yet the archegonial nucleus is relatively small. Fig. 35 is that of the actual contact, and shows clearly that in the meantime the female nucleus has markedly increased in volume. Fertilization in *Saxe-Gothaea* was not met with in the material worked through by Looby and Doyle (1939). Stages are available just before pollen tube entry and showing fusion just completed. From a comparison of these, and in view of the recognized changes mentioned in *Phyllocladus*, it is quite safe to accept for *Saxe-Gothaea* a ratio as in *Phyllocladus* or perhaps *Pherosphaera*. Lawson's (1932 a) account of *Microcachrys* is very fragmentary, but judging from his Fig. 28 of a "mature" archegonium there can hardly at fertilization be any great difference between the fusing nuclei which at most may be as in *P. nivalis* or may even approach subequality.

Data for *Dacrydium* are scanty and conflicting. Sinnott (1913) records that at maturity the female nucleus in *D. cupressinum* "almost fills the top of the archegonium." His figure, however, for *Dacrydium* (Pl. 5, fig. 3) is of *D. Bidwillii*, which, as already mentioned, is probably a *Podocarpus* closely related to the *Stachycarpus* forms. From the context it must be assumed that Sinnott considered the condition in the true *Dacrydium*, *D. cupressinum*, to be essentially similar and so there would be here a case of gross inequality at least as great as in *Podocarpus andinus*. Stiles (1911, figs, 3, 4), however, figures for *D. Colensoi* an archegonium with a pollen tube containing a male cell complex to all appearance approaching maturity but associated with an archegonium containing quite a small nucleus. If in *D. Colensoi* there were to be at maturity an egg nucleus "almost filling the top

of the archegonium " it is hard to see how Stiles could have missed it in preparations such as those yielding his Figs 3 and 4, even if it had not yet reached its full size. Thus there may be within *Dacrydium* a contrast of extremes as in *P. andinus* and *P. nivalis*, but it must be remembered that Sinnott's drawing is of an archegonium not apparently reached by any pollen tube. Such archegonia, as the ovule further matures, quite commonly show a large increase in size in unfertilized nuclei. This may be a case in point. Obviously, pending a critical re-examination of several species *Dacrydium* can only be introduced into podocarp discussion with great caution, if at all. Indeed this lack of accurate knowledge about so many features in *Dacrydium*, and the conflict in existing data, are a great handicap to a proper objective consideration of tendencies within the family as a whole.

On the basis of the foregoing only the most tentative suggestions can be made and then merely to focus attention on the problem. The condition of gross inequality could be basal in the family stock as it is apparently in the Pinaceae. It is, however, a constant in the Pinaceae whereas, if basal in the Podocarpaceae, it must have become modified to a simple marked inequality independently in each of the diverging lines within the family. Since, however, the *Saxe-Gothaea* and *Phyllocladus* types seem to show so many other features which may be reputed to be basal and primitive there is, on the data, no objection at least to considering the condition of more or less marked inequality, as there shown, to be the basal family feature. This feature would then have passed with little modification into four of the genera, including for the time being *Microcachrys*. Here too it may be of interest to recall that *Cephalotaxus*, whose gametophyte and archegonia otherwise so closely resemble the *Saxe-Gothaea-Phyllocladus* type, also shows the condition of simple marked inequality. On this view the major changes would be limited to *Podocarpus* and perhaps *Dacrydium*. Within *Podocarpus* two tendencies would then be shown. In the stachycarpean branch, arising early and retaining many primitive features, a gross inequality developed, paralleled possibly in the later *Dacrycarpus* section. The basal condition would have been retained in *Nageia*, but in *Eupodocarpus* arose the tendency to greater equality producing the *P. nivalis* type. If gross inequality were characteristic of the basal *Podocarpus* stock the appearance of the condition of mere marked inequality in *Nageia* would seem to imply a double change—from simple marked inequality to gross inequality in the *Podocarpus* stock, and then back to simple marked inequality. For this reason it is suggested that the development of gross inequality could be considered to be something special within the *Podocarpus* line whose stock, however, carried the common podocarpean feature of simple general inequality. The development would be essentially parallel for that already suggested for shallow and embedded archegonia.

The position is here set out rather to present the problem than to solve it. Knowledge of conditions in *Acmopyle* could well be critical. If this genus falls in with the *Phyllocladus-Pherosphaera-Saxe-Gothaea* group it would be confirmation of the basally primitive nature of the condition of simple marked inequality in the fusing nuclei of podocarps.

This brief review of a few features of comparison in podocarpean gametophytes, brought to notice by certain marked differences between *P. nivalis* and *P. andinus*, has perhaps done little more than focus attention on the numerous gaps in our knowledge concerning the details of gametophytic form and development in the Podocarpaceae, gaps which other workers may be able to fill. It has permitted, however, some tentative suggestions with regard to the nature of the gametophytes which may have been primitive in the basal stock and the nature of the gametophytic developmental tendencies possibly shown in the family. The basal stock must be old, so that the living seven genera must, most safely, be regarded as the end representatives of diverging lines, diverging perhaps from very early times. It is not now considered practicable to enquire whether, say, *Phyllocladus* is more closely related to *Microcachrys* than to *Pherosphaera*. In the group any such horizontal phylogeny is useless guesswork without a vertical phylogeny based on accurate fossil data. But, if gametophytic and embryonic features are reasonably conservative it may be possible, by a comparative examination of the living forms, to abstract as it were the common and so arrive at an idea of their basal primitive nature, and also to appreciate the developmental tendencies shown within the group. These tendencies need not be limited in their expression in any clean cut way to any genus but may appear here and there, perhaps varying even markedly in their degree of expression within a genus or even a group of species. The picture of gametophytic and embryological tendencies arrived at must primarily be considered as of interest *per se* in the sense that it can only be introduced into questions of ordinary systematy with great caution, if at all. Thus *Saxe-Gothaea*, though showing, for instance, great reduction in the axillary scalar part of the strobilus units and also a very specialized pollination behaviour resulting in wingless grains, nevertheless in the total features of its gametophytes and early embryogeny would appear closer to the basal type than any other living podocarp.

The results of this comparative survey can, however, best be summarized after embryogeny has been considered in a subsequent communication when a continuous picture of a basal podocarpean life history can be attempted.

Summary.

Development of the gametophytes, the male from pollination and the female from the first appearance of the rudiment of the ovule, is followed in *P. nivalis* up to the completion of fertilization, thus giving a reasonably full account of these stages in a typical member of the *Eupodocarpus* section. Earlier records are confirmed, corrected where necessary, and extended. Comparison is made with the full account already published of these stages in *P. andinus*, representing the *Stachycarpus* group. Comparison is also made with corresponding stages in other podocarps, as far as information on these are available. Preliminary tentative suggestions are made concerning the nature of the basal gametophytes and the gametophytic developmental tendencies in the family.

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